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Authors: Appleton, C. C., and Miranda, N. A. F.

Source: African Invertebrates, 56(1) : 1-17

Published By: KwaZulu-Natal Museum

URL: <https://doi.org/10.5733/afin.056.0102>

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Two Asian freshwater snails newly introduced into South Africa and an analysis of alien species reported to date

C. C. Appleton^{1*} and N. A. F. Miranda²

¹School of Life Sciences, University of KwaZulu-Natal, Westville Campus, P. Bag X54001, Durban, 4000 South Africa

²DST/NRF Research Chair in Shallow Water Ecosystems, c/o Department of Zoology, Nelson Mandela Metropolitan University, P.O. Box 77000, Port Elizabeth, 6031 South Africa

*Corresponding author: appletonc@ukzn.ac.za

ABSTRACT

Two Asian freshwater gastropods, *Radix rubiginosa* (Michelin, 1831) (Lymnaeidae) and *Gyraulus chinensis* (Dunker, 1848) (Planorbidae) are reported from a facility supplying fish and plants to the aquarium trade in KwaZulu-Natal, South Africa. Neither species has been found in South Africa previously. Both are considered potentially invasive. Updated keys for Lymnaeidae and *Gyraulus* spp. in South Africa are presented. An analysis of data on the dates of first collection and regions of origin for all 14 freshwater snails known to have been introduced into the country, regardless of whether or not they have survived, shows three phases of introduction. These are (i) from North America between the 1940s and 1960s, (ii) from South America during the 1970s and 1980s and (iii) from Asia between the 1990s and the present. Further research may show two species long regarded as indigenous to also be introductions.

KEY WORDS: Aquarium trade, introduced snails, phases of introduction.

INTRODUCTION

Freshwater snails are continually being translocated around the world via the aquarium industry, particularly the trade in aquarium plants (Walker 1978; Madsen & Frandsen 1989; Cowie 1998; Letelier *et al.* 2007), and a proportion of the species introduced to any country is likely to become invasive. South Africa is no exception to this and 40% of the introduced species listed by Appleton (2003) have become invasive. Invasive snails may have other important consequences such as direct environmental and economic effects, and indirect effects that impact human and animal health. Recent research has shown how one such species, *Tarebia granifera* (Lamarck, 1822), can become extremely abundant, dominate benthic communities (Miranda *et al.* 2011b; Miranda *et al.* 2014a,b) and alter the functioning of environments it has invaded (Miranda *et al.* 2011a; Moslemi *et al.* 2012). Indigenous snail species in particular are being affected and displaced by *T. granifera* in South Africa (Miranda & Perissinotto 2012; Raw *et al.* 2013). There is evidence that another invasive, *Physa acuta* Draparnaud, 1805, can displace the snail host of urogenital schistosomiasis and interrupt transmission of the disease (Dobson 2004).

With the exception of an Australian and an East African species that have not survived, all freshwater snail species introduced to South Africa before the 1990s (Appleton 2003) originated in the Americas. Then in 1999 the southeast-Asian caenogastropod *Tarebia granifera* was discovered in a reservoir in northern KwaZulu-Natal province (Appleton & Nadasan 2002). This contribution records the introduction of two additional Asian species, *Radix rubiginosa* (Michelin, 1831) and *Gyraulus chinensis* (Dunker, 1848). We also analysed data on all alien freshwater gastropod species reported from South Africa in terms of their dates of first collection and regions of origin. No freshwater bivalves have been introduced into South Africa.

TABLE 1

Selected chemical and physical characteristics of the tank and plant-tray water in which *R. rubiginosa* and *G. chinensis* respectively were collected.

	<i>R. rubiginosa</i> – tanks (n=35)	<i>G. chinensis</i> – shallow plant trays (n=8)
Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	123–139	964–1081
Dissolved oxygen concentration ($\text{mg}\cdot\text{l}^{-1}$)	7.72–8.68	7.53–7.74
Dissolved oxygen saturation (%)	72.7–90.7	88.5–89.1
pH	8.17–8.35	7.17–7.69

MATERIAL AND METHODS

Collection locality

Radix rubiginosa and *Gyraulus chinensis* were collected at a breeding facility for tropical fish and aquarium plants at Amatikulu, 110 km northeast of Durban in northern KwaZulu-Natal (29°04'S 31°39'E, 14.4 m). Unlike other aquarium supply facilities in South Africa, the Amatikulu facility is a 'flow-through' system in which water is circulated through the fish-holding tanks (4.0 × 2.5 × 1.1 m) into drains and into a small reservoir. This reservoir drains into a channel leading to the lowest reaches of the Amatikulu River. *Tarebia granifera* and *R. rubiginosa* were found in all four habitat types: tanks, drains, reservoirs and channels. *Gyraulus chinensis* was only found in shallow trays 30 mm deep in which plants were stored. Selected chemical and physical characteristics of the water from tanks and trays were measured using a YSI model 556 MPS Water Logger (Table 1). Voucher specimens of *R. rubiginosa* and *G. chinensis* were deposited in the KwaZulu-Natal Museum under accession numbers NMSA W9311 and NMSA W9314 respectively.

Molecular analyses

Lymnaeid snails were collected from Amatikulu (n=12) and from Durban (n=13) (29°52'S 30°59'E) in February 2007. Specimens were preserved in ethanol prior to analyses. DNA was extracted and isolated from foot tissue with the DNeasy® extraction kit using the solid tissue protocol. Fragments of the cytochrome c oxidase subunit I (COI) mitochondrial DNA and the 16S mitochondrial ribosomal DNA were amplified



Fig. 1. *Radix rubiginosa* from Amatikulu (photos: H. Madsen).

by polymerase chain reaction (PCR) and sequenced using primers and protocols outlined by Folmer *et al.* (1994) and Remigio and Blair (1997) respectively. PCR conditions were: 1 min at 95°C, then 34 cycles at 95°C for 30 s, 43°C for 30 s and 72°C for 1 min, followed by 72°C for 5 min for COI; and 2 min at 95°C; then 35 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 30 s, followed by 72°C for 10 min for 16S. Sequencing was done at Inqaba Biotechnical Industries (Pretoria, South Africa) with an ABI 3730 Capillary Sequencer using Big Dye technology. Sequences were edited and aligned using the programs BioEdit Sequence Alignment Editor (Version 7.0.5.3; Hall 1999) and Clustal X (version 1.81; Thompson *et al.* 1997). Bayesian phylogenetic analyses were performed using MrBayes (version 3.1.1; Ronquist & Huelsenbeck 2003). Neighbour-joining (NJ) and maximum parsimony (MP) phylogenetic analyses were performed using MEGA (version 2.1; Kumar *et al.* 1993). The program MrModelTest (version 1.0; Nylander 2004) was used to select the most appropriate nucleotide substitution model for use in NJ and Bayesian likelihood analyses. Complete homologous sequences for the COI and 16S genes were obtained from Genbank. *Planorbis corneus* was used to root the COI tree and *Radix ovata*, *Lymnaea aulacospira* and *Lymnaea stagnalis* were used as sister groups to the groups of interest. The 16S tree was rooted with *Stagnicola elodes*, and *Radix rubiginosa*, *Radix quadrasi*, *Radix* sp. EEAR-Canada-2002, *Radix* sp. EEAR-Philippines-2002, *Lymnaea* sp. EEAR-Hawaii-2002, *Lymnaea* sp. EEAR-China-2002 and *Austropeplea viridis* were used as sister groups to the groups of interest.

RESULTS

Table 1 shows that the water in both tanks and trays at Amatikulu was well oxygenated and slightly alkaline. The water in the trays had a much higher conductivity than the tank water, probably due to fertiliser used on the plants. The pH was higher in the tank water.

Radix rubiginosa (Michelin, 1831) (Basommatophora: Lymnaeidae)

Figs 1, 2

Identification

The shape and coloration of the shell of *R. rubiginosa* is variable and in Africa it can be mistaken for the indigenous *Lymnaea natalensis*¹ Krauss, 1848, itself a variable species (Brown 1994). Nadasan (2011) used discriminant function analysis to show that both juvenile and adult *R. rubiginosa* (from Amatikulu) had larger, more broadly ovate shells than *L. natalensis* from a natural habitat in Glenwood, Durban, with a higher body whorl and narrower aperture, often with a nearly straight outer margin. The maximum recorded shell height and width for *R. rubiginosa* is 34 × 20 mm (Brandt 1974) whereas for *L. natalensis* it is 25 × 14.5 mm (Brown 1994). The shell of *R. rubiginosa* is also thicker than that of *L. natalensis*. Thickness measurements made by Nadasan (2011) 2–4 mm from the outer apertural margins of *R. rubiginosa* and *L. natalensis* (n=15 each) were 0.19–0.16 mm and 0.13–0.09 mm respectively. Thus the shell of *R. rubiginosa* grows nearly 10 mm higher and 5 mm broader than *L. natalensis* and is 0.05–0.07 mm (28.9–44.2%) thicker.

¹ The taxonomy of *Lymnaea s.l.* is poorly resolved. The species *natalensis* has traditionally been referred to *Lymnaea* and we follow this usage, but note that others have referred it to *Radix* (Correa *et al.* 2010).



Fig. 2. Shells of *Radix rubiginosa* from Amatikulu (photos: N. Miranda). Scale bars = 5 mm.

The anatomy of lymnaeids is remarkably homogeneous across the family and Nadasan (2011) failed to find any differences between the reproductive systems of *R. rubiginosa* and the indigenous *L. natalensis*. The reproductive system of South African *L. natalensis* was described by Pretorius and Van Eeden (1969).

Molecular analysis

Molecular analyses indicate that the lymnaeids from Amatikulu (RRA) conform to *Radix rubiginosa* (Fig. 3). The lymnaeid collected in Durban (LND) is the indigenous *Lymnaea natalensis* as supported by morphological characteristics according to Pretorius and Van Eeden (1969), Brown (1994) (see also the key to Lymnaeidae in South Africa below) and a phylogenetic study on the basis of the 18S rDNA gene (Nadasan 2011).

Parasitology

Radix rubiginosa is of medical and veterinary importance in its native southeast Asia where it serves as the intermediate host for a variety of trematodes. These include the fasciolid *Fasciola gigantica* Cobbold, 1855, the giant liver fluke of domestic stock; three schistosomes (blood flukes), *Schistosoma incognitum* Chandler, 1926, *Orientobilharzia harinasutai* Kruatrachue, Bhaibulaya & Harinasuta, 1965 and *Trichobilharzia macgrathi* Kruatrachue, 1968, parasites of various mammals and birds; and two echinostomes *Echinostoma malayanum* Leiper, 1911 and *Hypoderaeum conoideum* (Bloch, 1782) (Brandt 1974; Chitramvong *et al.* 1981; Bunnag *et al.* 1983; Woodruff & Upatham 1993; World Health Organization 2005). *Fasciola gigantica* is a parasite of ungulates, including domestic stock, but may also infect people as do *E. malayanum* and *H. conoideum*. The normal hosts of these echinostomes are ducks and rats respectively. None of the schistosomes is able to produce patent infections in exposed people, but their cercariae cause a sometimes severe dermatitis. *Radix rubiginosa* has also been shown experimentally to be susceptible to the nematode *Angiostrongylus malaysiensis* Bhaibulaya & Cross, 1971 which may also infect people (Dondero & Lim 1976). The discovery of *R. rubiginosa* in South Africa is therefore of veterinary importance since *F. gigantica* infection is a recognised problem in domestic stock in the country (Reinecke 1983). The only known snail host for *F. gigantica* in South Africa is *Lymnaea*

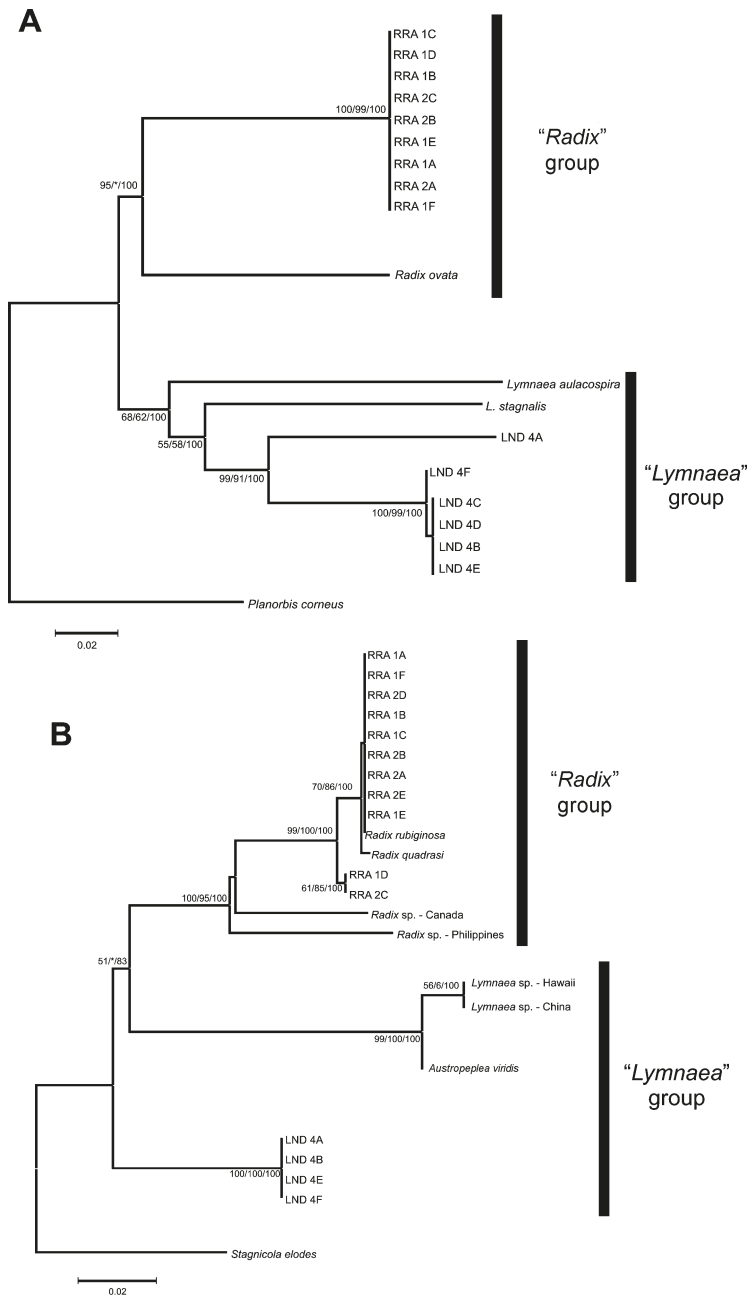


Fig 3. (A) Tree based on 533 nucleotides of the cytochrome c oxidase subunit I mtDNA of lymnaeid samples, with the outgroup *Planorbis corneus*. (B) Tree based on 360 nucleotides of the mt 16S rDNA of lymnaeid samples, with the outgroup *Stagnicola elodes*. Numbers on branches represent Bayesian posterior probabilities percentages, neighbour-joining and maximum parsimony bootstrap values, respectively. Only values greater than 50% are shown; * indicates support of < 50% for distance analysis. Figures adapted from Nadasan (2011). RRA – lymnaeid samples from Amatikulu, South Africa; LND – lymnaeid samples from Durban, South Africa.

natalensis, which occurs widely in both flowing and standing water bodies across the northern, eastern and southern parts of South Africa (De Kock *et al.* 2001). If *R. rubiginosa* is susceptible to the South African strain of *F. gigantica*, it could exacerbate the transmission of fascioliasis in the lowlands of KwaZulu-Natal and possibly further north as well.

Key to the Lymnaeidae in South Africa

Four lymnaeid species occur in South Africa; *L. natalensis*, *Galba truncatula* (O.F. Müller, 1774) and the invasive *Pseudosuccinea columella* (Say, 1817) and *R. rubiginosa*. They may be separated using the following key:

- 1 Shell ovate, basal whorl markedly swollen, spire usually much lower than the aperture.....3
- Shell slender, basal whorl not markedly swollen, spire usually not more than half the apertural height.....2
- 2 Shell with reticulate sculpture, spire up to half as high as aperture.....**Pseudosuccinea columella** (Say)
- Shell without reticulate sculpture, spire about as high as aperture**Galba truncatula** (O.F. Müller)
- 3 Mantle uniformly pigmented except for numerous small discrete spots, partially pigmented with small unpigmented spots and larger blotches, shell not robust, outer margin of basal whorl curved, aperture wide.....**Lymnaea natalensis** Krauss
- Mantle partially pigmented with small unpigmented spots and larger blotches, shell robust, outer margin of basal whorl nearly straight so that aperture is relatively narrow**Radix rubiginosa** (Michelin)

Gyraulus chinensis (Dunker, 1848) (Basommatophora: Planorbidae)

Figs 4, 5

Identification

Gyraulus chinensis is readily distinguished from the two indigenous species of *Gyraulus* in South Africa, *G. connollyi* Brown & Van Eeden, 1969 and *G. costulatus* (Krauss, 1848), by means of the characteristic dark pigmentation on its mid-dorsal mantle, head and tentacles, and its flatter and smoother shell (Brown *et al.* 1998; Brown 2001). In addition the shell of *G. chinensis* grows larger, to 6.5 mm (Brown *et al.* 1998), than either of the indigenous species, which rarely reach 5.0 mm (Brown & Van Eeden 1969). It is typically pale coloured with weak or no spiral sculpture and a rounded shoulder (Figs 4 and 5).

The pigmentation is seen on the head, anterior mantle and tentacles in both live and preserved specimens (Fig. 4). The ‘flatness index’ of a discoid shell was defined by Brown and Van Eeden (1969) and Brown *et al.* (1998) as the product of the whorl diameter from shoulder to the inside of the aperture and whorl height. The index increases with increasing whorl diameter. For *G. chinensis* from Amatikulu it ranged from 1.9 in juveniles of 1 mm diameter to 4.0 in adults of 4.5 mm diameter (Fig. 6) whereas in *G. connollyi*, the closest indigenous species (see key below), the whorls were higher relative to width so that the index was consistently below 3.0 (Brown & Van Eeden 1969). The two largest specimens of *G. chinensis* measured 5.65 × 1.4 and 5.75 × 1.35 mm

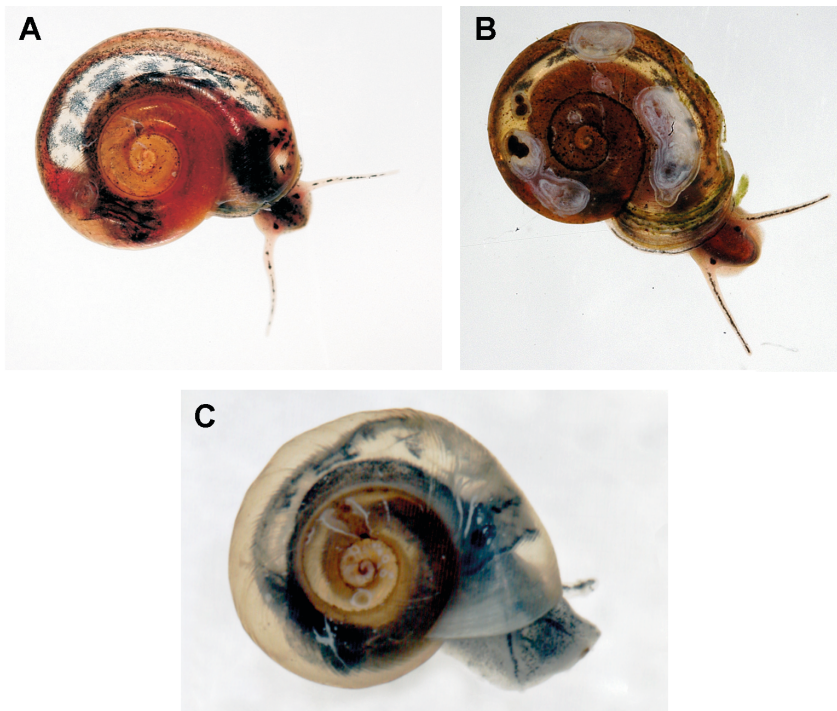


Fig. 4. *Gyraulus chinensis* from Amatikulu. Live specimens (4A–B, photos: H. Madsen) and specimen preserved in 70% ethanol (4C, photo: N. Miranda). The shell of the specimen in 4B shows patches of erosion. Note the pigmented mantle in both live and preserved specimens.

(Fig. 7). This figure shows that the number of whorls also increases with shell diameter from approximately 2.75 for shells less than 2 mm diameter to a maximum of 4.75 whorls for shells over 5.6 mm diameter.

The reproductive anatomy of the Amatikulu material conformed well to the descriptions of *G. chinensis* given by Brown *et al.* (1998) and Pointier (2008). Important here are the number and arrangement of the lobes of the prostate gland (12–17 lobes of uniform length) and the relative lengths of the preputium and penis sheath (preputium slightly shorter than penis sheath but similar in width). This differs from the two indigenous *Gyraulus* species, *G. connollyi* (Brown & Van Eeden 1969) and *G. costulatus* (Brown *et al.* 1998). These indigenous species are closely related and have a similar reproductive anatomy. They have fewer than 12 prostatic lobes, which are irregular in shape and arrangement. The preputium is usually longer than the penis sheath in both species though the widest part of the preputium is slightly broader than the penis sheath.

Key to species of *Gyraulus* in South Africa

One of the two indigenous species of *Gyraulus* in South Africa, *G. connollyi* is endemic to the central and southern parts of the country while the other, *G. costulatus*, is widespread across Africa (Brown 1994). *Gyraulus chinensis* may be separated from these two indigenous species by means of the following key:

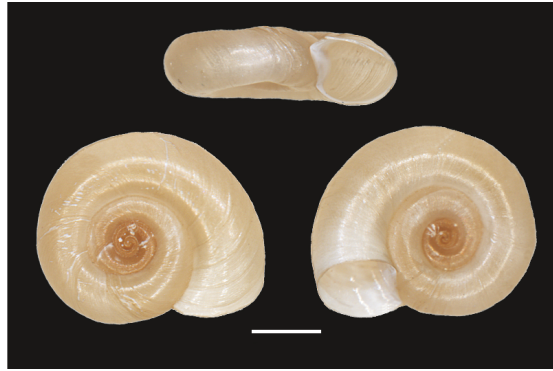


Fig. 5. Shell of *G. chinensis* from Amatikulu (photo: N. Miranda). Scale bar = 1 mm.

- 1 Shell distinctly ribbed, shoulder with an angular carina **costulatus** (Krauss)
- Shell not distinctly ribbed, shoulder rounded 2
- 2 Pigmentation of head, tentacles and mantle roof of pale spots and blotches, flatness index of whorl consistently below 3.0, prostate gland with 12 or more lobes of uniform length and arrangement, preputium usually longer than penis sheath **connollyi** Brown & Van Eeden
- Pigmentation of head, tentacles and mantle roof of conspicuous dark spots and blotches, flatness index of whorl between 1.9 and 4.0, prostate gland with fewer than 12 lobes, lobes of variable length and arrangement, preputium usually shorter than penis sheath **chinensis** (Dunker)

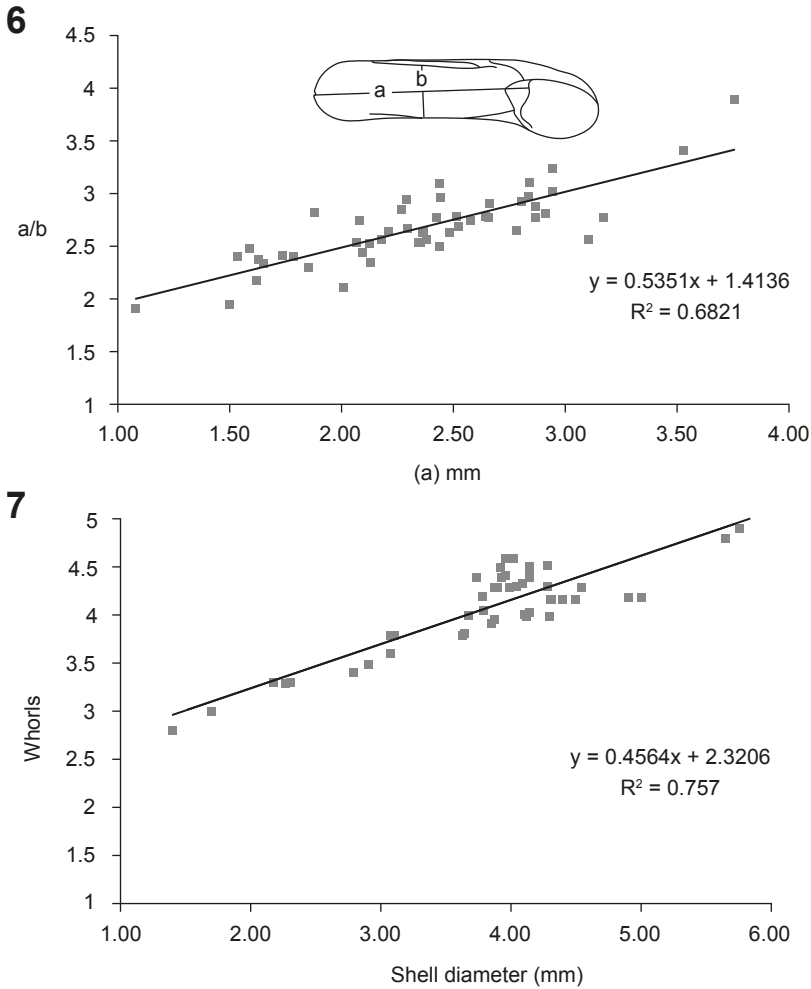
Parasitology

In its native region, *G. chinensis* serves as the first intermediate host for several trematodes, including two which may infect people: the fasciolid *Fasciolopsis buski* (Lankaster, 1857) reported from Thailand by Manning and Ratanarat (1970) and the echinostome *Echinostoma macrorchis* Ando & Ozaki, 1923 reported by Lo (1995) and the World Health Organization (1995).

DISCUSSION

Radix rubiginosa and *Gyraulus chinensis* are ‘hothouse’ or ‘greenhouse’ species, i.e. they originate in sub-tropical regions but have been introduced to and thrive in artificially heated habitats in cooler climates such as in Europe. In some situations they may spread from these artificial habitats.

Molecular analyses confirm the presence of *Radix rubiginosa* in Amatikulu (Fig. 3) and results are also in agreement with recent work by Correa *et al.* (2010). *Radix rubiginosa* was referred to as *Radix auricularia rubiginosa* by Brandt (1974) and was confirmed as a member of the *R. auricularia* (Linnaeus, 1758) complex by Remigio and Blair (1997). Correa *et al.* (2010) found that *R. rubiginosa* was most closely related to *R. quadrasi* (Möllendorff, 1898) from the Philippines. It is endemic to southeast Asia, viz. Malaysia, Thailand, Vietnam, Laos, Cambodia, Burma and Indonesia (Brandt 1974; Burch & Lohachit 1983).



Figs 6, 7. (6) Regression of shell flatness index (a/b) on (a) in mm for *G. chinensis* from Amatikulu; (7) Regression of number of whorls over shell diameter for *G. chinensis* ($n=53$) from Amatikulu. ($n=51$).

Radix rubiginosa is tolerant of a wide range of temperature, conductivity and pH, which may explain its occurrence in a variety of habitats such as rivers, lakes, ponds, rice fields and irrigation canals in its native region (Chitramvong *et al.* 1981). The only reported introductions of *R. rubiginosa* are isolated occurrences in aquaria in Israel (Mienis 1986), Britain and Ireland (Anderson, 2005) and in Australia where it was intercepted at Sydney airport in imported aquarium plants from Hong Kong (Walker 1978). In the same year Boray (1978) commented that *R. rubiginosa* could spread in Australia and 'have a wide distribution'. Dondero and Lim (1976), Mienis (2004) and Nadasan (2011) have all commented on how easy *R. rubiginosa* is to culture in aquaria and how quickly it becomes a pest. In such situations snails are likely to be discarded into nearby freshwater habitats. Since its discovery at Amatikulu in 2004, *R. rubiginosa* has been found in garden fish ponds in Durban 110 km to the south (C.C. Appleton,

TABLE 2
Snail species collected at aquarium plant and fish farm at Amatikulu.

Family	Species	Status in South Africa
Ampullariidae	<i>Pomacea diffusa</i>	Introduced, not invasive
Thiaridae	<i>Melanoides tuberculata</i>	Indigenous but may be being replaced by an invasive morph from Asia (Genner <i>et al.</i> 2004) or a hybrid
Thiaridae	<i>Tarebia granifera</i>	Invasive
Lymnaeidae	<i>Radix rubiginosa</i>	Introduced, status unknown
Planorbidae	<i>Gyraulus chinensis</i>	Introduced, potentially invasive
Planorbidae	<i>Helisoma duryi</i>	Established, not invasive
Physidae	<i>Physa acuta</i>	Invasive

unpublished data). If *R. rubiginosa* spreads in KwaZulu-Natal, it could compete with the indigenous *Lymnaea natalensis*. Indeed, recent experimental work by Nadasan (2011) on the bionomics of *R. rubiginosa* suggests that its invasive potential is similar to that of *Physa acuta*, the most widely distributed invasive freshwater snail in the world.

Gyraulus chinensis is endemic over a wide area of southern Asia from New Guinea, Indonesia and China in the east to Sri Lanka, India and Iran in the west (Meier-Brook 1983, 1984). Over this vast area it occurs as what Meier-Brook (1983) calls a 'Rassenkreis' which he defines as 'a polytypic species with a number of identifiable races that are not reproductively isolated'.

Evidence for the invasive potential of *G. chinensis* is provided by the distribution records given by Brown *et al.* (1998). These show that it was introduced to and has become invasive in Western Europe. Although it was found on many occasions in aquaria and greenhouses, it has, more importantly, been found since 1973 in rice fields and natural waterbodies in five European countries. Brown *et al.* (1998) also recorded its presence in rice fields in Guinea-Bissau, West Africa, in 1989, and its interception in South Africa in January 1995 by staff of the National Department of Agriculture's Directorate of Plant and Quality Control in a consignment of aquarium plants from Singapore. These latter snails were identified by Dr C. Meier-Brook (Institut für Tropenmedizin der Universität Tübingen, Germany) 'as a Rassenkreis of *Gyraulus chinensis*' (K.N. de Kock pers. comm. 2005). Recently the species has been collected in different types of habitat in different hemispheres: a pond in South Australia (Brown 2001), greenhouses in the Czech Republic (Beran & Glöer 2006) and a swamp in Martinique, West Indies (Pointier 2008). The present report is, however, the first of *G. chinensis* from inside South Africa and suggests that it may have become established. In 2004 *G. chinensis* was found in an aquarium in the foyer of a building on Howard College Campus, University of KwaZulu-Natal, Durban, South Africa, and traced to a collection of aquatic plants provided by the same commercial aquarium fish and plant breeder referred to under *R. rubiginosa*, i.e. at Amatikulu 110 km north of Durban, KwaZulu-Natal. Additional specimens were collected on visits to this facility on 17 August and 5 October 2005, 9 July 2008 and 18 January 2013. They were plentiful in shallow trays containing clear water ± 30 mm deep in which pots of aquatic plants are

TABLE 3

Updated list on introduced freshwater snail species in South Africa arranged according to their date of first collection. The earliest published source for each species in South Africa is also given.

Species	Year of first collection	Earliest published source	Region of origin
<i>Pseudosuccinea columella</i>	1942	Barnard (1948)	North America
<i>Physastra gibbosa</i>	1944	Barnard (1948) as <i>Lenameria dispar</i>	Australia
<i>Physa acuta</i>	1956	Van Bruggen (1966)	North and South America
<i>Biomphalaria angulosa</i>	1957	Mandahl-Barth (1957)	East Africa
<i>Helisoma</i> sp.	1960s	Van Bruggen (1974)	North America
<i>Helisoma duryi</i>	1966	Brown (1967)	North America
<i>Biomphalaria glabrata</i>	1969 and again in 1982 (see text)	Bruton & Merron (1985) as <i>Biomphalaria</i> sp.; Joubert <i>et al.</i> (1986) as <i>Biomphalaria</i> cf. <i>glabrata</i>	South America
<i>Pomacea diffusa</i>	1980s	Appleton (2003) as <i>P. bridgesi</i>	South America
<i>Marisa cornuarietis</i>	1986	Van Aardt & De Kock (1991)	South America
<i>Aplexa marmorata</i>	1986	Appleton <i>et al.</i> (1989)	South America and Caribbean islands
<i>Tarebia granifera</i>	1999	Appleton & Nadasan (2002)	Southeast Asia
<i>Radix rubiginosa</i>	2004	This paper	Southeast Asia
<i>Gyraulus chinensis</i>	2006	This paper	Southern Asia

stored for sale, and on the submerged parts of the plants themselves. These plants are sold (airfreighted) to other provinces of South Africa and also to Europe and elsewhere. It should therefore be expected that *G. chinensis* and/or its eggs laid on leaves will be (or have been) translocated to other areas as well, as they were to the aquarium at the University of KwaZulu-Natal.

It is not known how or precisely when *R. rubiginosa* and *G. chinensis* were introduced but the fact that plants from the same source as that examined here, and on which *G. chinensis* was found, are airfreighted around the country and as far afield as Europe raises the possibility that the snails have been introduced to aquaria and ornamental ponds over a wide area. It should therefore be expected to spread to natural systems in South Africa as well. The discoveries of *R. rubiginosa* and *G. chinensis* focus attention on the aquarium trade, whose imports into South Africa are poorly regulated. Seven snail species from five families have been found in the various habitats at the fish farm sampled here (Table 2). All but one of these are alien to South Africa; two of them have become invasive and at least one is potentially invasive. The single non-alien is *Melanooides tuberculata* (O. F. Müller, 1774) (see below).

The three non-Asian species in Table 2, *Pomacea diffusa* Blume, 1957 (recorded as *P. bridgesi* by Appleton 1996, 2003), *Helisoma duryi* (Wetherby, 1879) and *Physa acuta*,

commonly occur in aquaria worldwide and are to be expected wherever the aquarium trade is practised. Hayes *et al.* (2008) concluded that the species of *Pomacea* commonly distributed via the aquarium trade is *P. diffusa* whereas the notoriously invasive *P. canaliculata* (Lamarck, 1819) has been distributed for food, largely in Asia. *Ampullaria lineata*, recorded by Appleton (1996), is a misidentification of the wild-type of *P. diffusa*. It is, however, noteworthy that the three most recent additions to the South African list of introduced freshwater snails, *Tarebia granifera* (1999), *G. chinensis* (2004) and *R. rubiginosa* (2006), all originated in southeast Asia. It is significant that while seven species occur at the fish farm, *Pseudosuccinea columella*, the most widely distributed invasive snail in South Africa (De Kock *et al.* 1989) does not. The fact that *P. columella* does not occur in southeast Asia either (Brandt 1974; Burch & Lohachit 1983) while the others do, supports an Asian origin via the aquarium trade for the species listed in Table 2.

Table 3 presents an updated list of 13 species known to have been introduced to South Africa with estimated dates of first collection and regions of origin. The approximate rate of introduction over the 72 years since the earliest reported introduction in 1942 is one new species every 5½ years—similar to the rate estimated for introduced terrestrial gastropods by Herbert (2010) though over a much shorter time period. We estimate that 76.9% (10/13) were introduced via the aquarium or ornamental plant trade. Of the remaining three (*Biomphalaria angulosa* Mandahl-Barth, 1957, *B. glabrata* (Say, 1818) and *Marisa cornuarietis* (Linnaeus, 1758)), two, *B. glabrata* and *M. cornuarietis*, were introduced for schistosomiasis research purposes, but no explanation can be offered for the entry of *B. angulosa* into South Africa. *Biomphalaria glabrata* (Fig. 8) was in fact introduced twice, first to a man-made canal in central Durban, probably during the 1960s, but the population had died out by the mid-1980s (J. Agnew pers. comm. July 1983; Bruton & Merron 1985; Ashton *et al.* 1986). Its shells measured up to 26.5 × 8.3 mm (n=16) with 6 to 7 whorls. It is thus much larger than any African *Biomphalaria* species including *B. angulosa* (Brown 1994) and also larger than most Neotropical species except *B. glabrata* and *B. tenagophila* (Orbigny, 1835), both of which commonly reach 30 mm and are hosts for *Schistosoma mansoni* Sambon, 1907 in Brazil (Malek 1985; Anon. 2008). Joubert *et al.* (1986) reported it as *Biomphalaria* cf. *glabrata* and it proved susceptible to an Egyptian strain of *S. mansoni* (K.N. de Kock pers. comm. 2013). After examining photographs of the Durban shells, Dr J-P. Pointier (France) (in litt., 17.07.2014) and Dra S. Thiengo (Brazil) (in litt., 31.07.2014) supported Joubert's 1986 provisional identification with near certainty. Unfortunately no soft parts are available so this identification cannot be confirmed.

Biomphalaria glabrata was reintroduced, but to laboratories for schistosomiasis research purposes in 1982, the same year that Pflüger warned of the dangers of doing so (Pflüger 1982). This species is an efficient host of *S. mansoni* in Brazil and *M. cornuarietis*, introduced in 1986, is a herbivore which destroys the egg masses of other snails, including species of *Biomphalaria*, as it feeds. It was thus tested as a competitor/predator of the local schistosomiasis host snails. There is no evidence that either *B. glabrata* or *M. cornuarietis* have escaped or occur in natural systems in South Africa today.

Biomphalaria angulosa, an east African species that is susceptible to *Schistosoma mansoni*, was identified from 'near' Durban and in Oaklands, Johannesburg, by Mandahl-Barth (1957, 1958). Van Eeden *et al.* (1965) and Van Eeden (1966a, b) noted that the Johannesburg population existed for two years but had died out by 1957 as had the 'near'

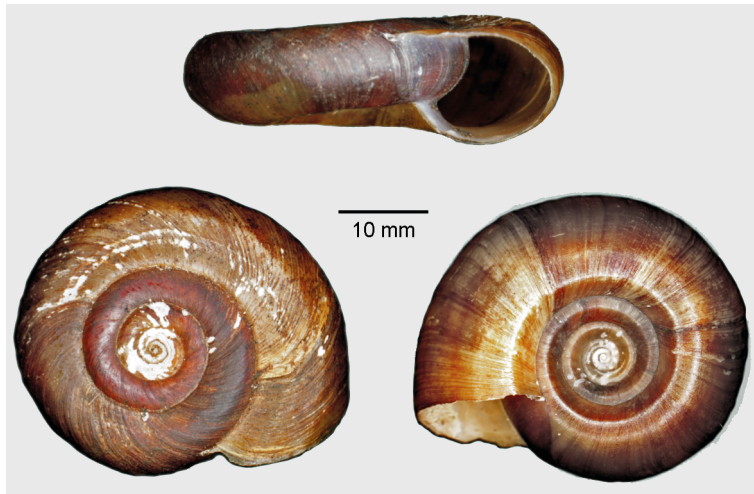


Fig. 8. Shell of *Biomphalaria glabrata* collected in Durban in the 1980s (photos: C. Appleton).

Durban population. Brown (1994) considered both reports to be doubtful and that the ‘near’ Durban record may have been a case of incorrect locality data.

Two species not included in Table 3 but which may have been introduced are *Melanoides tuberculata* (Table 2) and *Galba truncatula*. Both require molecular analysis to determine whether or not they were introduced. Although *M. tuberculata* is indigenous to South Africa (Connolly 1939; Brown 1994), the characteristic tuberculate morph illustrated by Van Eeden (1960), Oberholzer and Van Eeden (1967) and Appleton (1996) is rare in the country today. The morph currently common in Durban was illustrated by Pointier *et al.* (2003) and resembles the Asian genotype/morph shown by Genner *et al.* (2004) to have invaded Lake Malawi. This is the subject of ongoing investigation.

Van Eeden (1958, 1966a) proposed that *G. truncatula* had been introduced to South Africa from Europe though he (Van Eeden *et al.* 1965) drew attention to Connolly’s 1939 records of fossil and sub-fossil shells which suggest that the species previously had a wider distribution across the sub-continent. Recently, however, Correa *et al.* (2010) presented molecular evidence that *G. truncatula* originated in North America but has spread widely in recent times. This needs to be confirmed but it is possible that European colonists introduced *G. truncatula* to South Africa just as they did to the altiplano of Bolivia (Bargues *et al.* 1997).

Allowing for lag periods, the dates of first collection and regions of origin of the 14 species listed in Table 3 enable the delineation of three phases in introduction: species first collected between the 1940s and mid-1960s, originating mostly in North America, some perhaps coming via Europe; those first collected between the late 1960s and 1980s in South America; and those first collected more recently, between the 1990s and the present, in Asia. The arrival of *T. granifera* around 1996 appears to have heralded the Asian phase in the introduction of freshwater snails, including *R. rubiginosa* and *G. chinensis*, into South Africa.

General trends in South African trade data indicate that imports from Asia have essentially doubled during the period from 1998 (15% of total imports by value) to

2011 (29% of total imports by value), according to South African Revenue Service data on main trade partners for that period. South African records of newly introduced alien invasive gastropods from Asia coincide with this time period. As import frequency increases so does the possibility of alien species introduction. Better control is essential to prevent repeated and further introductions into South Africa (see Robinson 1999).

The spread of freshwater snails has frequently been attributed to the aquarium trade and aquarium plants in particular (Pointier 1999; Strayer 2010). *Gyraulus chinensis* was probably introduced to South Africa as a passenger on prohibited aquarium plants such as *Myriophyllum spicatum* L. coming from Malaysia and Indonesia, i.e. following the major South African aquarium plant import trade line. It is difficult to track alien species introductions into South Africa via pet stores, aquarists and the internet-mediated trade; however, Martin and Coetzee (2011) report that a variety of invasive and/or prohibited plants are traded within South Africa and that there is a general lack of knowledge about identification and regulation of alien species. As alien species are continuously moved and traded in the country, recurring local introductions (propagule pressure) will increase and contribute to their establishment and spread. This surely accounts for the appearance of *Aplexa marmorata* (Guilting, 1828) in the southwestern Cape, more than 1200 km from KwaZulu-Natal and Mpumalanga where it has thus far been confined (Mlambo *et al.* 2011). A census of snails in aquarium supply facilities across the country is needed.

The findings of this report draw attention to the ongoing accidental importation of alien aquatic invertebrates into South Africa via the poorly regulated aquarium trade. There is a clear need to improve the regulation of this trade and to educate customs officers and auditors on the 'best practice' recommendations for controlling such introductions as outlined by Appleton and Bosman (2000). These include an illustrated database of potentially invasive species and increased public awareness of the harmful effects of invasive organisms in invaded ecosystems (see also Robinson 1999).

The National Lists of Invasive Fresh-water Invertebrates published in the South African *Government Gazette* of 12 February 2014 are related to the National Environmental Management Biodiversity Act (NEMBA) (Act No. 10 of 2004). List 9 (National List of Invasive Fresh-water Invertebrate Species, p. 45) lists three snail species, *Aplexa marmorata*, *Pseudosuccinea columella* and *Tarebia granifera*, all of which are discussed in the present report. Others listed as invasive in Table 3 should be included. List 10 (prohibited fresh-water invertebrates, p. 72) lists four bivalve species that would undoubtedly present threats should they be introduced. These are *Corbicula fluminea* (O.F.Müller, 1774), *Dreissena polymorpha* (Pallas, 1771), *Dreissena rostriformis bugensis* (Andrusov, 1897) and *Potamocorbula amurensis* (Schrenck, 1861).

ACKNOWLEDGEMENTS

We are grateful to Guy Upfold for permission to collect snails at Amatikulu and to Dr Dai Herbert (Kwa-Zulu-Natal Museum) for help with literature. Prof. K.N. de Kock (Northwest University), Dr H. Madsen (University of Copenhagen) and Kuvendren Pillay (University of KwaZulu-Natal) are also thanked for their contributions of lymnaeid samples, photographs and unpublished molecular data respectively. Dr J-P Pointier (Université de Perpignan) and Dra S. Thiengo (Instituto Oswaldo Cruz) confirmed the identity of *Biomphalaria glabrata* from Durban.

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